nonsymbiotic hemoglobin functions as a facilitator of oxygen diffusion in dividing cells and as an oxygen sensor to meet increased demand for oxidativ respiration. The Examiner has further taken the position that Bailey teaches a method for improving the agronomic properties of a plant comprising providing a plant with increasing intracellular oxygen binding protein. The Examiner has then taken the position that combining Andersson and Bailey teaches a method of using nonsymbiotic hemoglobin to increase cellular oxygen in plants and thereby improving agronomic properties of the plant.

It is respectfully requested that the Examiner reconsider this rejection.

Regarding Andersson, applicant notes that Andersson proposes multiple functions for nonsymbiotic plant hemoglobins. These include acting as a sensor of oxygen concentration (paragraph 1, line 3), acting as a facilitator of oxygen diffusion at low oxygen concentrations (paragraph 1, lines 12-13), acting in oxygen transport (paragraph 1, line 14), acting as a facilitator of oxygen diffusion in dividing cells(paragraph 2, lines 2-6), being associated with high levels of metabolic activity (paragraph 2, lines 6-10), and facilitating intracellular diffusion of oxygen to the mitochondria (paragraph 2, lines 15-18). Regarding the Examiner's comments, applicants note that it is clearly shown in the instant application as filed that the non symbiotic plant hemoglobins are not involved in mitochondrial oxidation (page 12, lines 7-25) and that they also do not facilitate diffusion of oxygen (page 22, lines 3-5). Rather, they are involved in the binding of oxygen under low oxygen environments and maintaining cell en rgy status. Thus an individual taking the disclosure of Andersson t al

and attempting to show that the non symbiotic hemoglobin was acting in mitochondrial oxidative phosphorylation or facilitating the diffusion of oxygen would note that increased levels of the non symbiotic plant hemoglobins had no effect. The skilled artisan would therefore conclude that the non symbiotic plant hemoglobins were having no effect and that there would be no agronomic benefit from expressing them in plant tissues.

Regarding Bailey, applicants note that Bailey describes the importance of increased intracellular oxygen levels as the functional mode of action of the *Vitreoscilla* hemoglobin (see for example, Bailey page 4, lines 6 and 11 and page 6, lines 19, 23 and 27). Bailey also notes the particular suitability of a hemoglobin with high k_{off} rates or low oxygen affinity. As discussed in the application as filed and in the response filed November 30, 2001, the nonsymbiotic plant hemoglobins have <u>low</u> k_{off} rates. Furthermore, the nonsymbiotic hemoglobins have high oxygen avidity, not low oxygen affinity as is the case with horse heart myoglobin, another target listed by Bailey as "particularly suitable". Thus, Bailey teaches against the use of nonsymbiotic plant hemoglobins as these proteins have very different properties, specifically, vastly different oxygen binding characteristics compared to those described by Bailey and are clearly not functionally equivalent. Furthermore, based on the properties of the nonsymbiotic hemoglobins, one of skill in the art, in view of Bailey, might conclude that overexpression of nonsymbiotic hemoglobin proteins would in fact restrict oxygen availability in a cell rather than increase it and would therefore have a negative impact on agronomic properties of a plant.

Thus, as discuss d above and as discussed in the response dated November 30, 2001, Bailey teaches against the use of nonsymbiotic hemoglobins because these proteins have significantly different oxygen binding properties than those described by Bailey. Thus, there would be no incentive for one of skill in the art to combine Bailey and Andersson as Bailey teaches that the non-symbiotic hemoglobins would not be suitable for improving agronomic properties of a plant. Even if one of skill in the art were to combine Andersson and Bailey, the resulting plants would show higher hemoglobin levels in root elongation, cotyledons and stem but no effect would be observed on oxygen diffusion or on oxidative respiration, as discussed above. Furthermore, as discussed on page 8, lines 21-26, when grown in an air environment, over expression of the nonsymbiotic plant hemoglobin had minimal effect on growth rate, oxygen consumption and cellular ATP levels. It was only once the plants were grown under nitrogen that the effect of the nonsymbiotic hemoglobins on ATP levels was noted. Thus, even if one of skill in the art were to combine Bailey and Andersson, (despite the fact that Bailey teaches that this combination would not work and may in fact restrict oxygen availability) no effect would be seen unless the plants were grown under a nitrogen atmosphere, which is not taught or suggested by either Andersson or Bailey.

Thus, Bailey in fact teaches that nonsymbiotic hemoglobins would not be suitable for improving agronomic properties of plants, meaning that there

would be no incentive for one of skill in the art to combine Bailey and Andersson. Even if Bailey and Anderss n were c mbined, one of skill in the art would have noted that over-expression of the nonsymbiotic hemoglobins had no effect on oxygen diffusion or on oxidative respiration and concluded that the nonsymbiotic hemoglobins of Andersson were not functional in the teachings of Bailey.

In view of the foregoing, further and more favorable consideration is respectfully requested.

Respectfully submitted
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